Drosophila development: **A receptor for ommatidial recruitment**

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Recent work shows that the differentiation of all the cell types found in the compound eye of *Drosophila melanogaster* is induced by reiterative activation of the EGF receptor.

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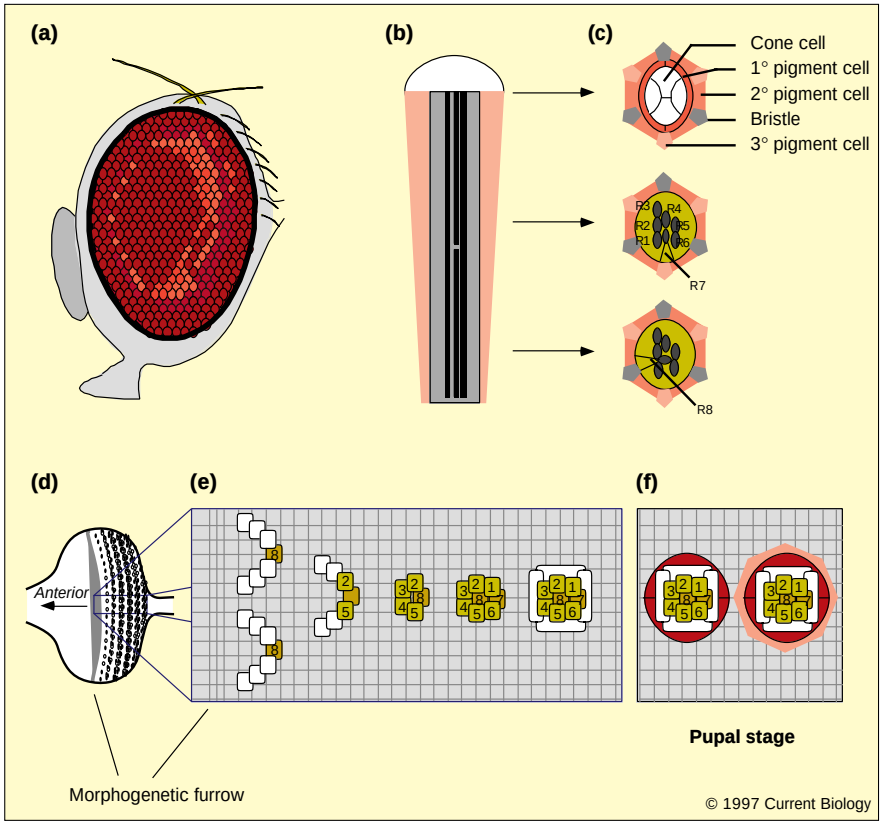
In recent years, studies on eye development in the fruitfly *Drosophila* have greatly contributed to our understanding of many biological issues, from the roles cell–cell interactions play in pattern formation to the nature of components that make up the intracellular signalling pathways downstream of receptor tyrosine kinases. As a result of many studies, the mechanisms that control the generation of a single photoreceptor (R7) have been largely unravelled, whereas the processes that generate the other cell types found in the eye have remained elusive. Now data have been obtained that support a simple model explaining the recruitment of all cell types found in the eye [1].

The *Drosophila* compound eye comprises about 750–800 single units, the facets or ommatidia, each containing 20 cells. Each ommatidium has six outer and two inner photoreceptors — R1–R6 and R7 and R8, respectively — which collect the visual input and transmit it to the optic lobes. Four cone cells secrete the lens, and two primary and six secondary and tertiary pigment cells optically insulate the individual ommatidia. Eye development starts during larval stages from an epithelial monolayer, the eye imaginal disc (Fig. 1). Up to the third larval stage, all eye imaginal disc cells divide. From then on, cell divisions occur in two waves, which sweep anteriorly across the eye disc. In between these zones of proliferation, a small groove known as the morphogenetic furrow sweeps anteriorly across the disc. Ommatidial development is initiated

Figure 1



The *Drosophila* eye (a) comprises 750–800 single ommatidia (b), each of which contains 20 cells. (c) The different cells, illustrated in these cross-sections at three different levels of an ommatidium, can be easily recognized by morphological and physiological criteria. (d) During larval stages, eye development is initiated in the eye imaginal disc. The morphogenetic furrow sweeps anteriorly across the disc, leaving more and more mature ommatidia behind. (e) The different stages of ommatidial development are depicted. Immediately posterior to the morphogenetic furrow, arc-like structures appear. At the top of each arc, the R8 cell is specified; subsequently, photoreceptor cells R2 and R5, and then R3 and R4 are added to form the five-cell precluster. The other ommatidial cells are recruited from the cells generated in the second mitotic wave, again in a sequential manner: first R1 and R6, then R7 followed by the four cone cells. (f) During pupal stages, the different pigment cells are added.

in the morphogenetic furrow, and as time progresses more and more complete developing ommatidia are left behind the morphogenetic furrow (Fig. 1; reviewed in [2]).

Just behind the morphogenetic furrow, the cell located at the top of an arc-like cell group is selected to become the future R8 cell. Subsequently, R2 and R5, as well as R3 and R4, are recruited to initiate neuronal development. The remaining uncommitted cells undergo a final round of cell division — the second mitotic wave. From the resulting pool of cells, the last three photoreceptors join the cluster: first R1 and R6, and then R7. Soon thereafter, four cone cells are added. As ommatidial development is sequential, at any given time during the third larval stage each developmental step is occurring at some point in the eye disc, from the earliest, recruitment step to the later steps where the cone cells are added to the cluster [2] (Fig. 1); the pigment cells join the photoreceptor clusters during pupal development.

The acquisition of the correct cell fate within an ommatidium does not depend on cell lineage, but rather involves cell–cell interactions. The positional cues read by the developing R cells are likely to be short-range signals released within the ommatidium. A specific 'combinatorial induction' model of photoreceptor cell development has been postulated, in which each cell acquires its fate by the unique combination of specific signals presented by its neighbours [2]. Genetic analyses of R7 cell development initially supported this model. The receptor tyrosine kinase Sevenless reads an inductive signal — the ligand Bride-of-sevenless (Boss) — presented by the R8 cell. Subsequent genetic screens revealed that the positional information read by the Sevenless receptor is transduced inside the cell by a conserved pathway involving the small GTP-binding protein Ras and a series of protein kinases, eventually reaching the nucleus (reviewed in [3]). Sevenless is not, however, sufficient to generate an R7 cell: it just triggers neuronal development in an already prepatterned cell [4]. Whereas Sevenless is required only for the development of the R7 cell, Ras signalling is required for all photoreceptor cells [3].

The only other receptor tyrosine kinase known to be involved in photoreceptor development is the *Drosophila* homologue of the epidermal growth factor (EGF) receptor (DER). From the phenotype associated with a *gain-of-function* allele of the *DER* gene, which leads to the *Ellipse* mutant eye phenotype, DER was first thought to be required for correct ommatidial spacing [5]. However, later studies using null alleles and mosaic animals — in which DER activity is normal in some cells but totally lacking in others — showed that DER is required for neuronal differentiation of all photoreceptor cells [6]. The role of DER during ommatidial development has recently been elucidated in some detail by Freeman [1], who assayed the effects of expressing dominant-negative and constitutively-activated forms of DER.

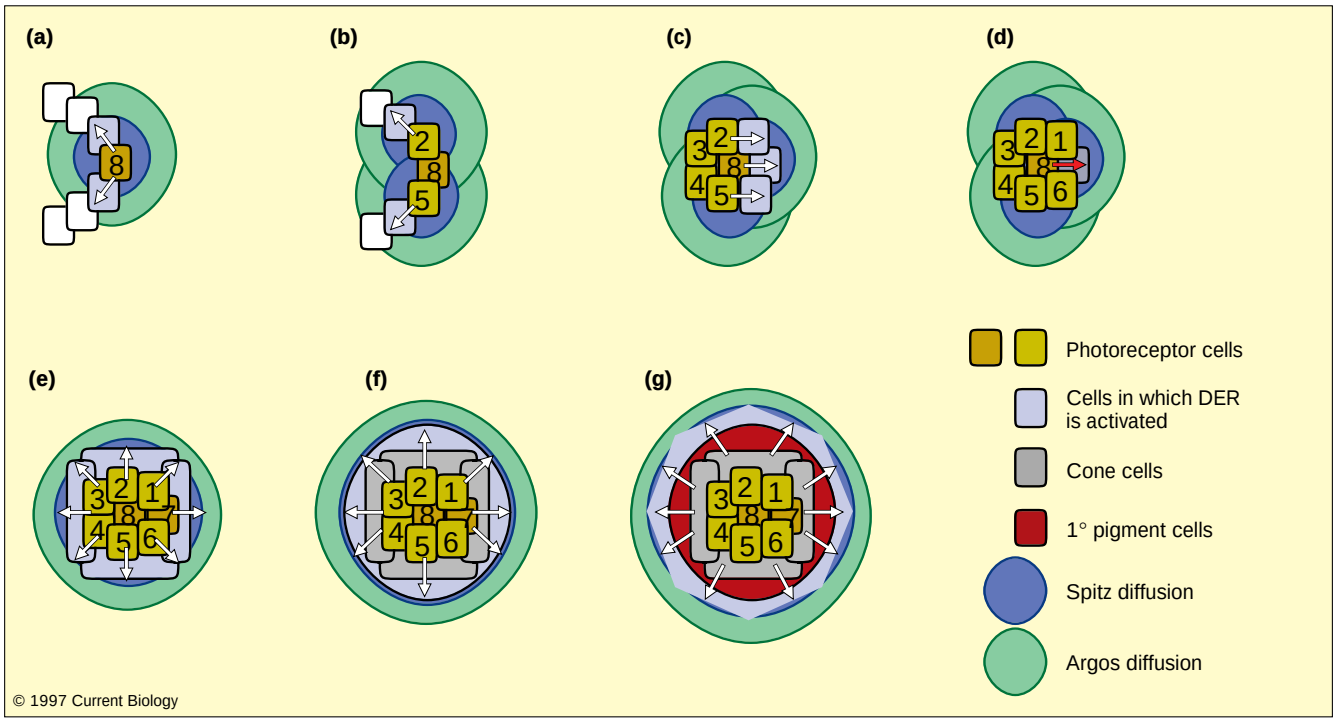
Freeman [1] found that expression of dominant-negative DER in all cells posterior to the morphogenetic furrow completely blocked the formation of ommatidia. The expression of dominant-negative DER for just a brief period, using an inducible promoter, resulted in the loss of those photoreceptor cells that were initiating development at that time. At later stages of ommatidial development, the induction of dominant-negative DER expression blocked the formation of cone and pigment cells, whereas the formation of photoreceptor cells was unaffected [1].

Having shown that all cells in the ommatidium depend on DER function for their formation, Freeman [1] went on to study the effects of the activated DER, in two ways: by overexpressing a constitutively-active DER protein; and by overexpressing the main DER ligand, the product of the *spitz* gene. The *spitz* gene encodes a protein related to the transforming growth factor α (TGF-α) family of secreted signalling molecules, which activates DER when it binds to the receptor following proteolytic processing. As expected from the results of expressing the dominant-negative DER, *spitz* expression resulted in the over-recruitment of all ommatidial cell types. Ubiquitous activation of DER *via* ectopic *spitz* expression is lethal for the fly, but some animals make it to the larval stages where they display a similar phenotype as flies carrying a dominant gain-of-function *DER* allele [5], supporting the notion that DER has an additional function in ommatidial spacing before its cell-recruitment function.

The recruitment of the R7 cell to the ommatidial precluster requires the action of two receptor tyrosine kinases, DER and Sevenless. Both receptor tyrosine kinases feed into the same signal transduction cascade and, as Freeman [1] showed, they appear to be fully interchangeable. Constitutively-activated DER or Sevenless proteins both induce the formation of extra R7 cells or outer photoreceptor cells [1,4]. Furthermore, expression of activated DER can rescue the *sevenless* mutant eye phenotype. The important message to be taken from Freeman's experiments is thus that the recruitment of different ommatidial cell types appears to depend on the timing of receptor tyrosine kinase activation. R7 development is special, as it requires two bursts of Ras activation, the first mediated by DER and the second by Sevenless. Indeed, some neuronal characteristics are activated in the presumptive R7 cell independently of Sevenless [7].

This work complements earlier studies on the function of the gene *argos*. Freeman and others have identified *argos* as a negative regulator of cell-fate decisions in the eye. The *argos* gene encodes a secreted protein with an EGF-like domain, which diffuses several cell diameters further

Figure 2



A model for the control of photoreceptor cell recruitment, as recently suggested by Freeman [1]. The first cell selected is R8, in a process that depends on the proneural gene *atonal*. R8 then successively recruits flanking cells by activation of DER signalling (open arrows) under the combined control of the antagonistic proteins Spitz (blue) and Argos (green). **(a)** Surrounding the R8 cell, a zone of high Spitz concentration (blue) recruits R2 and R5. More remote cells are inhibited from initiating neuronal differentiation by Argos (green), which antagonizes DER signalling. **(b)** R2 and R5 now secrete Spitz and Argos, and in turn recruit R3 and R4. Only cells in the arc are competent to respond to the inductive signals; arc cells not integrated into the cluster (mystery cells) are finally excluded from the ommatidial cluster. **(c)** Following the second mitotic wave, the five-cell precluster recruits R1 and R6. **(d)** The presumptive R7 cell is routed towards a neural fate as well, but requires a second burst of Ras activation *via* Boss and Sevenless (red arrow) to be recruited to the forming ommatidium. **(e)** The R cells produce Spitz and Argos, and the flanking cells, in which DER is subsequently activated, are recruited as cone cells. **(f,g)** Additional rounds of recruitment occur during pupal stages to add the different pigment cells. The model implies that the specification of the cell types recruited to the cluster depends on the timing of DER activation.

than the *spitz* product. The Argos protein competes with Spitz for binding to DER, and acts as an inhibitor of the DER. The *argos* mutant phenotype is equivalent to the phenotype caused by DER activation, involving the over-recruitment of all ommatidial cell types. Conversely, over-expression of wild-type *argos* results in the recruitment of fewer cells to the ommatidial cluster, just like expression of dominant-negative DER. Most importantly, *argos* was shown to be activated by DER signalling, and thus closes a negative-feedback loop that limits the strength and duration of DER signalling ([8] and references therein).

R8 is the first ommatidial cell selected in a process requiring the proneural gene *atonal* [9]. Neuronal development of this cell depends on Ras, but is initiated even in the absence of *spitz* [10]. The action of DER, its activating ligand Spitz and the negative regulator Argos led Freeman [1] to delineate a simple model for the control of subsequent cell recruitment in the *Drosophila* eye (Fig. 2). As the R8 cell differentiates, it produces two diffusible proteins that modulate DER activity: the Spitz protein diffuses over only a few cell diameters and activates DER in neighbouring cells; the Argos protein diffuses further, and represses DER activity in more remote cells. Thus, the initial R8 cell is surrounded by cells with activated DER; these start to differentiate as neuronal cells and subsequently secrete both Spitz and Argos. The different diffusion rates of the two proteins create a zone of DER activation immediately surrounding the neuronal precluster. The iterative use of this principle finally results in the formation of the complete ommatidium (Fig. 2). A similar recruitment mechanism has very recently been shown to be used in the developing embryonic peripheral nervous system [11]. As reported by zur Lage *et al.* [11] in this issue of *Current Biology*, the first precursor cells of the stretch receptors — known as chordotonal organs — are specified by the proneural gene *atonal*, and additional chordotonal organ precursor cells are subsequently recruited by activation of the DER signalling cascade in neighbouring cells.

The beauty of the model presented by Freeman [1] is that it explains the recruitment of all the different cell types in the retina without the need to introduce specific signals and receptors. It leaves us, however, with some open and important questions. How are the different cell types specified? Do cells somehow count the time elapsed before they are triggered to differentiate [12]? How do undifferentiated cells influence ommatidial recruitment? The finding that the gene *fat facets*, which encodes an ubiquitin-specific protease, is required in undifferentiated cells [13] to control the number of photoreceptor cells suggests that the one-way flux of information as depicted in Figure 2 is an over-simplification, and there are other mechanisms still to be discovered and incorporated into the model.

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